



Year: 2009

Amino acetate functionalized Schiff base organotin(IV) complexes as anticancer drugs: synthesis, structural characterization, and in vitro cytotoxicity studies

Basu Baul, T S ; Basu, S ; De Vos, D ; Linden, Anthony

Abstract: Potassium 2-[(2Z)-(3-hydroxy-1-methyl 2-butenylidene)]amino-4-methyl-pentanoate (L1HK) and potassium 2-[(E)-1-(2-hydroxyphenyl)alkylidene]amino-4-methyl-pentanoates (L2HK-L3HK) underwent reactions with $\text{Ph}_n\text{SnCl}_{4-n}$ ($n = 2$ and 3) to give the amino acetate functionalized Schiff base organotin(IV) complexes $[\text{Ph}_3\text{SnLH}]_n$ (1-3) and $[\text{Ph}_2\text{SnL}]$ (4), respectively. These complexes have been characterized by ^1H , ^{13}C , ^{119}Sn NMR, IR spectroscopic techniques in combination with elemental analyses. The crystal structures of 1 and 3 were determined. The crystal structures reveal that the complexes exist as polymeric chains in which the L-bridged Sn-atoms adopt a trans- R_3SnO_2 trigonal bipyramidal configuration with the Ph groups in the equatorial positions and the axial locations occupied by a carboxylate oxygen atom from one carboxylate ligand and the alcoholic or phenolic oxygen atom of the next carboxylate ligand in the chain. The carboxylate ligands coordinate in the zwitterionic form with the alcoholic/phenolic proton moved to the nearby nitrogen atom. The solution structures were predicted by ^{119}Sn NMR spectroscopy. When these organotin(IV) complexes were tested against A498, EVSA-T, H226, IGROV, M19 MEL, MCF7 and WIDR human tumor cell lines, the average ID50 values obtained were 55, 80 and 35 ng/ml for triphenyltin(IV) compounds 1-3, respectively. The most cytotoxic triphenyltin(IV) compound in the present report (3) with an average ID50 value of around 35 ng/ml is found to be more cytotoxic for all the cell lines studied than doxorubicin, cisplatin, 5-fluorouracil and etoposide.

DOI: <https://doi.org/10.1007/s10637-008-9189-1>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-21185>

Journal Article

Published Version

Originally published at:

Basu Baul, T S; Basu, S; De Vos, D; Linden, Anthony (2009). Amino acetate functionalized Schiff base organotin(IV) complexes as anticancer drugs: synthesis, structural characterization, and in vitro cytotoxicity studies. *Investigational New Drugs*, 27(5):419-431.

DOI: <https://doi.org/10.1007/s10637-008-9189-1>

Amino acetate functionalized Schiff base organotin(IV) complexes as anticancer drugs: synthesis, structural characterization, and in vitro cytotoxicity studies

Tushar S. Basu Baul · Smita Basu · Dick de Vos · Anthony Linden

Received: 31 July 2008 / Accepted: 30 September 2008 / Published online: 22 October 2008
© Springer Science + Business Media, LLC 2008

Summary Potassium 2-{[(2*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}-4-methyl-pentanoate (L^1HK) and potassium 2-{[(*E*)-1-(2-hydroxyphenyl)alkylidene]amino}-4-methyl-pentanoates (L^2HK - L^3HK) underwent reactions with Ph_nSnCl_{4-n} ($n=2$ and 3) to give the amino acetate functionalized Schiff base organotin(IV) complexes $[Ph_3SnLH]_n$ (1–3) and $[Ph_2SnL]$ (4), respectively. These complexes have been characterized by 1H , ^{13}C , ^{119}Sn NMR, IR spectroscopic techniques in combination with elemental analyses. The crystal structures of 1 and 3 were determined. The crystal structures reveal that the complexes exist as polymeric chains in which the L-bridged Sn-atoms adopt a *trans*- R_3SnO_2 trigonal bipyramidal configuration with the Ph groups in the equatorial positions and the axial locations occupied by a carboxylate oxygen atom from one carboxylate ligand and the alcoholic or phenolic oxygen atom of the next carboxylate ligand in the chain. The carboxylate ligands coordinate in the zwitterionic form with the alcoholic/phenolic proton moved to the nearby nitrogen atom. The solution structures were predicted by ^{119}Sn NMR spectroscopy. When these organotin(IV) complexes were

tested against A498, EVSA-T, H226, IGROV, M19 MEL, MCF7 and WIDR human tumor cell lines, the average ID_{50} values obtained were 55, 80 and 35 ng/ml for triphenyltin (IV) compounds 1–3, respectively. The most cytotoxic triphenyltin(IV) compound in the present report (3) with an average ID_{50} value of around 35 ng/ml is found to be more cytotoxic for all the cell lines studied than doxorubicin, cisplatin, 5-fluorouracil and etoposide.

Keywords Anti-cancer drugs · Organotin(IV) · Amino acetate functionalized Schiff bases · Potassium 2-{[(2*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}-4-methyl-pentanoate · Potassium 2-{[(*E*)-1-(2-hydroxyphenyl)alkylidene]amino}-4-methyl-pentanoates · Cell lines · NMR · Crystal structure

Introduction

One of the most important goals of pharmacological research is the search for new molecular structures which exhibit effective antitumour activities [1–2]. This has driven inorganic and organometallic chemists to look for new metal compounds with good activities, preferably against tumours that are responsible for high cancer mortality. Organotin(IV) compounds are a widely studied class of metal-based antitumour drugs and their intensive investigation has led to the discovery of compounds with excellent in vitro antitumour activity, but, in many cases, disappointingly low in vivo potency or high in vivo toxicity [3–5]. It is well established that organotin(IV) compounds are very important in cancer chemotherapy because of their apoptotic inducing character [6, 7]. The design of improved organotin(IV) antitumour agents occupies a significant place in cancer chemotherapy, as revealed from their

T. S. Basu Baul (✉) · S. Basu
Department of Chemistry, North-Eastern Hill University,
NEHU Permanent Campus, Umshing,
Shillong 793022, India
e-mail: basubaul@hotmail.com

D. de Vos
Pharmachemie BV,
P.O. Box 552, 2003 RN Haarlem, The Netherlands

A. Linden (✉)
Institute of Organic Chemistry, University of Zurich,
Winterthurerstrasse 190,
8057 Zurich, Switzerland

remarkable therapeutic potential reflected in recent research reports [8–19].

The binding ability of organotin(IV) compounds towards DNA depends on the coordination number and nature of the groups bonded to the central tin atom. The phosphate group of DNA sugar backbones usually acts as an anchoring site and the nitrogen of DNA base binding is extremely effective, this often resulting in the stabilization of the tin center as an octahedral species. Low doses of organotins can exhibit anti-tumoural activity [20–25] and have suggested an action mode via a gene-mediated pathway in the cancer cells, opening a new research sub-area on organotin(IV) compounds. The chemical and biochemical aspect of DNA inhibition, including biotechnological aspects of organotin(IV) cancer chemotherapy, has also been described [26].

Organotin(IV) halides and their complexes with amines and other ligands exhibit borderline activities in vivo against P388 and L1210 leukaemia. The in vivo pre-screenings against these two cancers used initially by the National Cancer Institute (NCI), USA, were later replaced by in vitro pre-screenings against a panel of human tumour cell lines, viz., MCF-7 and EVSA-T (mammary cancers), WIDR (colon cancer), IGROV (ovarian cancer), M19 MEL (melanoma), A498 (renal cancer) and H226 (non-small-cell lung cancer) [27].

When organotin(IV) halides are dissolved in water, the pH of the solution decreases dramatically because they are converted slowly into organotin(IV) hydroxides and then to bis(triorganotin)oxides or diorganotin oxides. In contrast, di- and tri-organotin(IV) carboxylates do not suffer from this disadvantage and generally remain intact in water for long periods; i.e. days. Consequently, a large number of organotin(IV) carboxylates have been investigated for their antitumour potential. Among organotin(IV) carboxylates, triorganotin(IV) carboxylates are quite well known as bactericides and fungicides [28, 29] and subsequently several such derivatives were found to be potent when screened for their cytotoxicity [30–32]. Exceptionally high in vitro antitumour activities were also reported for triphenyltin(IV) benzoates and salicylates against a human mammary tumour (MCF-7) and a colon carcinoma (WIDR) and found to be comparable with that of mitomycin C [33]. Several attempts were also made to synthesize triphenyltin(IV) carboxylates by modifying the carboxylate moiety with biologically active carboxylate moieties and also by incorporating lipophilic/hydrophilic properties in them, since the lipophilic properties are essential for crossing the cell membrane and their hydrophilic character for being accepted by the water-rich cell [27]. The promising development in the search for antitumour organotin(IV) compounds has been achieved with some triphenyltin(IV) carboxylates, such as 3,6-dioxaheptanoate and 3,6,9-

trioxadecanoate [34], 4-carboxybenzo-15-crown-5 and 4-carboxybenzo-18-crown-6 [34, 35], steroidcarboxylate [36] and terebate [27, 32, 37] when screened in vitro against human tumour cell lines, as per the NCI protocol.

In view of the increasing interest in organotin(IV) carboxylates and prompted by their structural diversity [38] and broad therapeutic activity [27], organotin(IV) complexes of Schiff bases derived from amino acids have also been investigated extensively [39–49]. An overview of the coordination behaviour of such Schiff bases towards organotin(IV) is shown in Scheme 1. Some of these organotin(IV) compounds were screened for antitumour activity in vivo in Ehrlich ascites carcinoma cells [41] and cytotoxic activity in vitro against cell lines of human origin [47, 49]. The in vitro cytotoxicity results demonstrated that triphenyltin(IV) compounds derived from 2-{[(2Z)-(3-hydroxy-1-methyl-2-butenylidene)]amino}-, 2-{[(E)-1-(2-hydroxyphenyl)methylidene]amino}- and 2-{[(E)-1-(2-hydroxyphenyl)ethylidene]amino}-phenylpropionates are more active than CDDP (cisplatin) [49]. Interestingly, all three triphenyltin(IV) compounds show comparable cytotoxic activity across a panel of cell lines and this prompted us to investigate related systems by modifying the amino acetate part of the molecule, which might improve dissolution properties and thereby influence cytotoxicity. Within this paper, we present a series of new organotin(IV) carboxylates involving the 2-{[(2Z)-(3-hydroxy-1-methyl-2-butenylidene)]amino}-4-methyl-pentanoate and 2-{[(E)-1-(2-hydroxyphenyl)-alkylidene]amino}-4-methyl-pentanoate skeletons (Scheme 2, compounds 1–4), their synthesis, spectroscopic characterization, crystal structures and preliminary cytotoxic studies.

Experimental

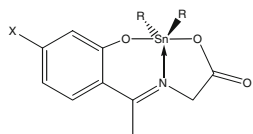
Materials

Ph₃SnCl (Fluka AG), Ph₂SnCl₂, 2'-hydroxyacetophenone (Aldrich), *l*-leucine (Himedia), 2-hydroxybenzaldehyde and acetylacetone (Sisco) were used without further purification. The solvents used in the reactions were of AR grade and were dried using standard procedures.

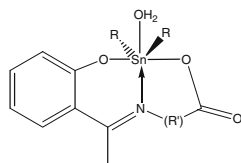
Physical measurements

Carbon, hydrogen and nitrogen analyses were performed with a Perkin Elmer 2400 series II instrument. IR spectra in the range 4,000–400 cm⁻¹ were obtained on a Perkin Elmer Spectrum BX series FT-IR spectrophotometer with samples

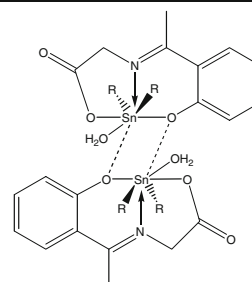
Scheme 1 An overview showing the coordination behaviour of Schiff bases with amino acids towards the organotin(IV) moiety ►



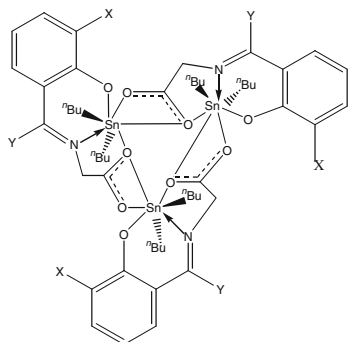
(R = C₆H₅, X = H; R = C₆H₅, X = CH₃)

I

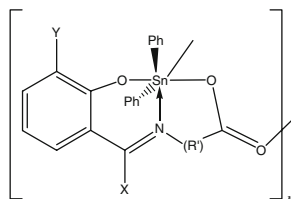
(R = CH=CH₂, R' = CH₂; R = ⁿC₄H₉, X = CHCH₂C₆H₅)

II

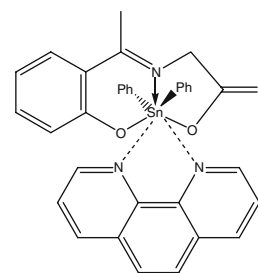
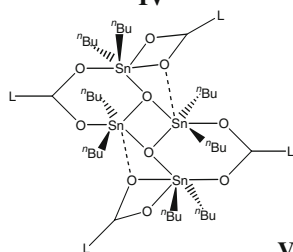
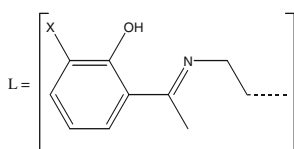
(R = CH₃ or ⁿC₄H₉)

III

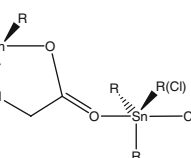
(X = H, Y = H; X = CH₃, Y = CH₃)

IV

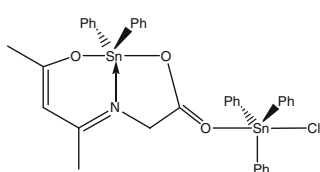
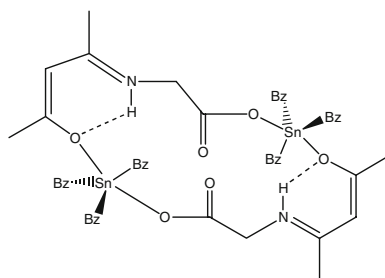
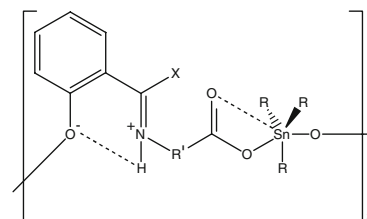
(X = CH₃ or H, Y = CH₃ or H, R' = CH₂ or CHCH₂C₆H₅)

V**VI****VII**

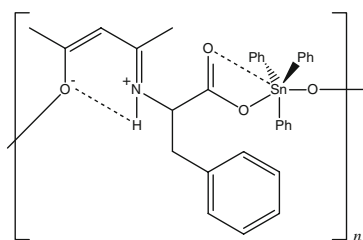
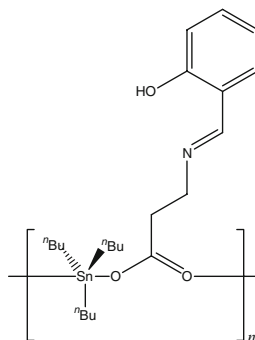
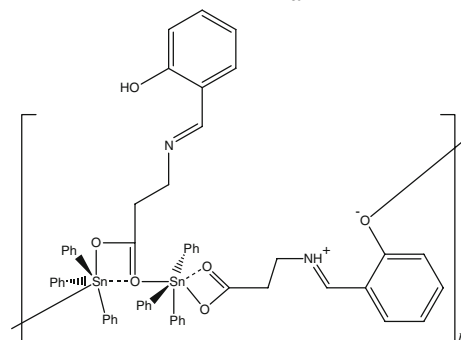
(X = H or CH₃)

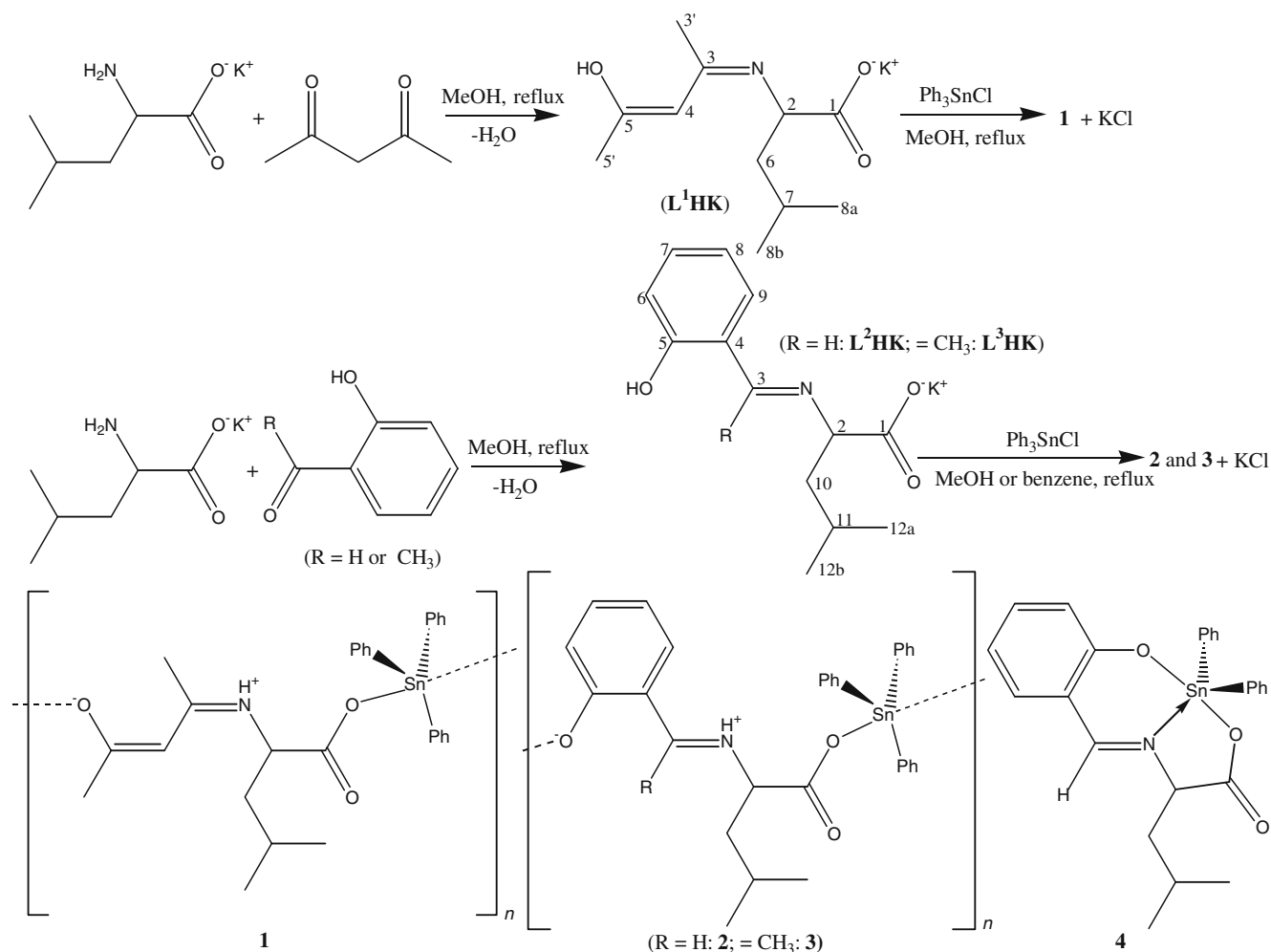


(R = ⁿC₄H₉, X = H; R = C₆H₅, X = H; R = C₆H₅, X = CH₃)

VIII a**VIII b****IX**

(R = CH₃ or C₆H₅, R' = CH₂, X = CH₃ or H;
R = C₆H₅, R' = CHCH₂C₆H₅, X = CH₃ or H)

X a**X b****XI****XII**



Scheme 2 Syntheses of potassium salts (L^1HK – L^3HK) along with the numbering scheme and their triphenyltin(IV) complexes (1–3). The structure of diphenyltin(IV) complex (4) is included for the convenience of discussion

investigated as KBr discs. The 1H -, ^{13}C - and ^{119}Sn -NMR spectra were recorded on a Bruker AMX 400 spectrometer and measured at 400.13, 100.62 and 149.18 MHz. The 1H , ^{13}C and ^{119}Sn chemical shifts were referred to Me_4Si set at 0.00 ppm, $CDCl_3$ set at 77.0 ppm and Me_4Sn set at 0.00 ppm, respectively.

X-ray crystallography

Crystals of compounds **1** and **3** suitable for an X-ray crystal-structure determination were obtained from toluene/hexane (v/v 1:1) and ethanol, respectively, by slow evaporation of the solutions of the respective compounds. All measurements were made on a Nonius KappaCCD diffractometer [50] with graphite-monochromated $Mo\ K\alpha$ radiation ($\lambda = 0.71073\ \text{\AA}$) and an Oxford Cryosystems Cryostream 700 cooler. Data reduction was performed with HKL Denzo and Scalepack [51]. The intensities were corrected for Lorentz and polarization effects, and empirical absorption correc-

tions based on the multi-scan method [52] were applied. Equivalent reflections, other than Friedel pairs, were merged. The data collection and refinement parameters are given in Table 1, and views of the molecules are shown in Figs. 1 and 2. The structure of **1** was solved by direct methods using SIR92 [53]. Heavy-atom Patterson methods [54] were employed for **3**, which revealed the position of the Sn-atom, and the remaining non-hydrogen atoms in **3** were located in a Fourier expansion of the Patterson solution, which was performed by DIRDIF94 [55].

Both triphenyltin(IV) compounds exist as polymeric chains with the carboxylate ligands bridging between the Sn-atoms and in each case the asymmetric unit contains just one of the chemical repeat units of the polymer. One of the phenyl ligands in **1** and **3** and the *iso*-propyl group in **1** are disordered over two orientations. Two sets of overlapping positions were defined for the atoms of the disordered groups and the site occupation factors of the major conformations of these groups refined to 0.578(9), 0.552(8)

Table 1 Crystallographic data and structure refinement parameters for organotin(IV) compounds 1 and 3

	1	3
Empirical formula	C ₂₉ H ₃₃ NO ₃ Sn	C ₃₂ H ₃₃ NO ₃ Sn
Formula weight	562.18	598.22
Crystal size (mm)	0.20×0.25×0.30	0.10×0.12×0.17
Crystal shape	Prism	Prism
Temperature (K)	250(1)	160(1)
Crystal system	Orthorhombic	Monoclinic
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁
<i>a</i> (Å)	9.2766(1)	9.3293(2)
<i>b</i> (Å)	14.0307(2)	13.6667(3)
<i>c</i> (Å)	21.2691(2)	11.1178(2)
β (°)	90	91.713(1)
<i>V</i> (Å ³)	2768.33(6)	1416.89(5)
<i>Z</i>	4	2
<i>D_x</i> (g cm ^{−3})	1.349	1.402
μ (mm ^{−1})	0.950	0.933
Transmission factors (min, max)	0.769; 0.834	0.829; 0.914
2 θ_{\max} (°)	55	55
Reflections measured	74188	32799
Independent reflections; <i>R</i> _{int}	6331; 0.072	6469; 0.056
Reflections with <i>I</i> >2 σ (<i>I</i>)	5409	5819
Number of parameters	401	393
Number of restraints	251	170
<i>R</i> (<i>F</i>) [<i>I</i> >2 σ (<i>I</i>)reflns]	0.036	0.046
<i>wR</i> (<i>F</i> ²) (all data)	0.084	0.114
GOF(<i>F</i> ²)	1.09	1.11
$\Delta\rho_{\max, \min}$ (e Å ^{−3})	0.98; −0.40	1.43; −0.75

and 0.70(1), respectively. Similarity restraints were applied to the chemically equivalent bond lengths and angles involving all disordered C-atoms, while neighbouring atoms within and between each conformation of the disordered groups were restrained to have similar atomic displacement parameters.

The imine H-atom in 1 was placed in the position indicated by a difference electron density map and its position was allowed to refine together with an isotropic displacement parameter. All remaining H-atoms were placed in geometrically calculated positions and refined by using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to 1.2 U_{eq} of its parent C-atom (1.5 U_{eq} for the methyl groups). The refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\sum w(F_o^2 - F_c^2)^2$. Corrections for secondary extinction were applied. Two reflections in 3, whose intensities were considered to be extreme outliers, were omitted from the final refinement. Refinement of the absolute structure parameter [56] for 1 and 3 yielded the values of −0.02(2) and −0.05(3), respectively, which confidently confirm that the refined coordinates represent

the true enantiomorph. All calculations were performed using the SHELXL97 program [57].

Several crystals of 2 were tried, but all seem to exhibit diffuse scattering. Although the overall structure could be discerned and is very similar to that of 3, the *R*-factors remained very high, the refinement is unstable because of pseudosymmetry, and there is some unresolvable disorder at the chiral centre of the *l*-leucine ligand. For these reasons, the details of this structure are not reported here. Visual inspection of the crystals showed that they tended to grow in layered blocks. An individual layer could be isolated by careful cutting, but this did not yield improved data. It is possible that twinning has occurred, although twin analysis software did not reveal any twin laws and reconstructed precession images were also reasonably clean. Data collection at various temperatures did not improve the results.

Synthesis

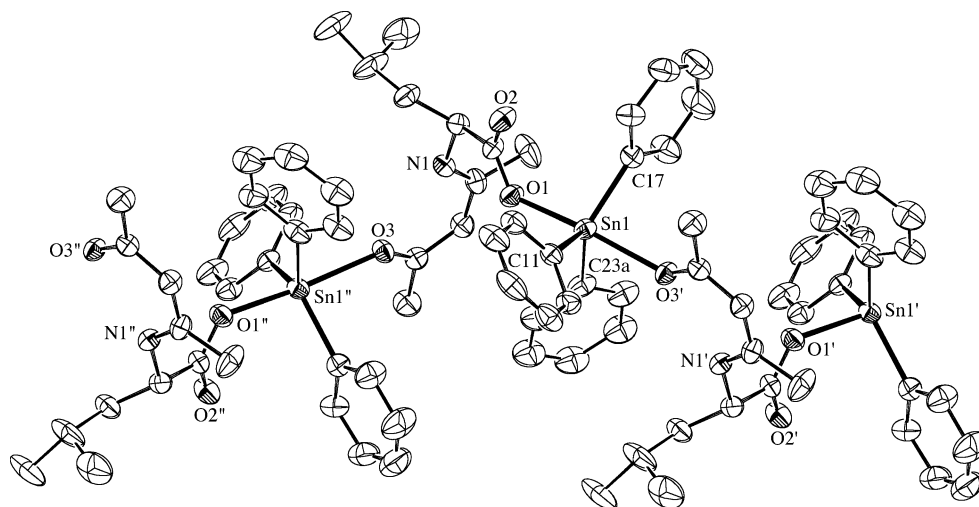
Synthesis of the potassium salts

A typical procedure is described below.

Potassium 2-{[(2*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}-4-methyl-pentanoate (L¹HK) was prepared by slow addition of a methanol solution (2 ml) of KOH (0.19 g, 3.38 mmol) to *l*-leucine (0.44 g, 3.35 mmol) in 10 ml methanol with continuous stirring. A methanolic solution (15 ml) of acetylacetone (0.33 g, 3.29 mmol) was added drop-wise. A pale yellow colour developed almost immediately and stirring was continued for 1 h, followed by 5 h refluxing. The volatiles were removed carefully; the pale yellow mass was stirred with diethylether and filtered. The residue was dissolved in the minimum amount of anhydrous methanol and filtered. The filtrate was precipitated with diethylether which afforded the crude product. Repeated precipitations from a methanol-diethylether mixture yielded pure L¹HK, which was then dried in vacuo (0.68 g, 81% yield). M.p.: 70–72°C. Anal. Calc. for C₁₁H₁₈NKO₃: Theory: C, 52.56; H 7.21; N; 5.57%. Found: C, 52.50; H, 7.16; N, 5.52%. IR absorptions (cm^{−1}): 1,672 $\nu(\text{OCO})_{\text{asym}}$, 1,606 $\nu(\text{C=N})$, 1,301 $\nu(\text{Ph(C-O)})$.

The other potassium salts (Scheme 2), viz., potassium 2-{[(*E*)-1-(2-hydroxyphenyl)methylidene]amino}-4-methyl-pentanoate (L²HK) and potassium 2-{[(*E*)-1-(2-hydroxyphenyl)ethylidene]amino}-4-methyl-pentanoate (L³HK) were prepared analogously by reacting 2-hydroxybenzaldehyde and 2'-hydroxyacetophenone, respectively, with potassium *l*-leucinate. L²HK: Recrystallized from methanol to give a bright yellow precipitate in 83.7% yield. M.p.: 153–55°C. Anal. Calc. for C₁₃H₁₆NKO₃: Theory: C, 57.11; H, 5.90; N, 5.12%. Found: C, 57.03; H, 5.88; N, 5.10%. IR absorptions (cm^{−1}): 1,639 $\nu(\text{OCO})_{\text{asym}}$, 1,613 $\nu(\text{C=N})$, 1,374 $\nu(\text{Ph(C-O)})$. L³HK: Recrystallized from methanol to

Fig. 1 Three repeats of the crystallographically and chemically unique unit in the polymeric $[\text{Ph}_3\text{SnL}^1\text{H}]_n$ chain structure of **1** (50% probability ellipsoids). The H-atoms and one of the disordered conformations have been omitted for clarity



give a yellow precipitate in 78.7% yield. M.p.: 145–47°C (decomp.). Anal. Calc. for $\text{C}_{14}\text{H}_{18}\text{NKO}_3$: Theory: C, 58.51; H, 6.31; N, 4.87%. Found: C, 58.40; H, 6.20; N, 5.04%. No meaningful IR spectrum could be recorded owing to fast decomposition of the sample.

Synthesis of the organotin(IV) complexes

Synthesis of $[\text{Ph}_3\text{SnL}^1\text{H}]_n$ (**1**)

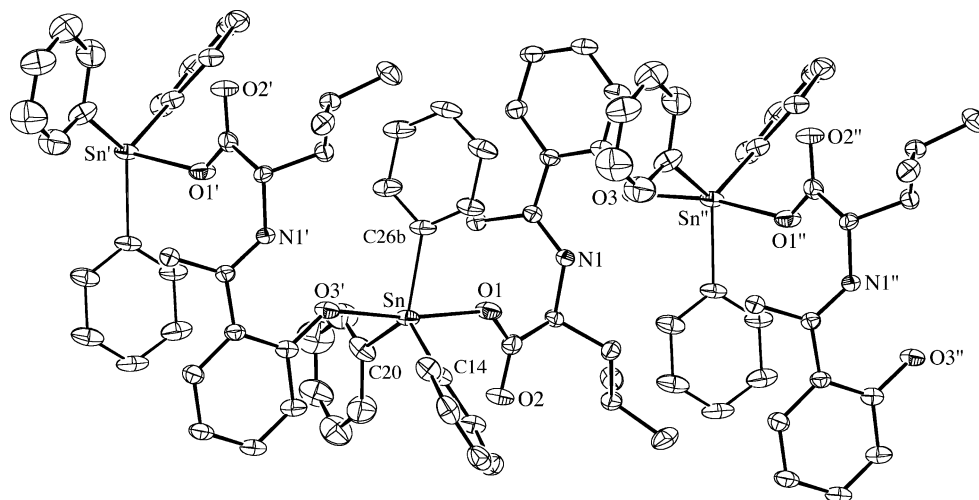
Ph_3SnCl (0.55g, 1.43 mmol) in anhydrous methanol (ca. 10 ml) was added drop-wise to a stirred anhydrous methanol solution (ca. 20 ml) containing L^1HK (0.36g, 1.43 mmol). The solution was refluxed for 5 h at ambient temperature and the volatiles were removed in vacuo. The residue was washed thoroughly with hexane, filtered and dried in vacuo. The residue was extracted into anhydrous benzene and filtered. The benzene solution was concentrated to a minimum and precipitated with hexane. The precipitate was washed several times with hexane, dried in vacuo and recrystallized from toluene-hexane mixture (1:1 v/v) to yield

colourless crystals of **1** in 83.5% (0.66g) yield. M.p.: 135–137°C. Anal. Calc. for $\text{C}_{29}\text{H}_{33}\text{NO}_3\text{Sn}$: Theory: C, 61.95; H, 5.92; N, 2.49%. Found: C, 61.90; H, 5.85; N, 2.40%. IR absorptions (cm^{-1}): 1,646 $\nu(\text{OCO})_{\text{asym}}$, 1,600 $\nu(\text{C}=\text{N})$, 1,261 $\nu(\text{Ph}(\text{CO}))$. $^1\text{H-NMR}$ (CDCl_3): Ligand skeleton: 10.92 (brs, 1H, OH), 4.97 (s, 1H, H-4), 4.18 (d (7.7 Hz); $^3J(^{119/117}\text{Sn}-^1\text{H}=22\text{ Hz})$), 1H, H-2), 2.01 (s, 2H, H-6), 1.74 (s, 6H, H-3' and H-5'), 1.59 (s, 1H, H-7), 0.91 and 0.85 (d (7 Hz), 6H, H-8a and H-8b); Sn-Ph skeleton: 7.78 (m, 6H, H-2*), 7.45 (m, 9H, H-3* and H-4*), ppm. $^{13}\text{C-NMR}$ (CDCl_3): Ligand skeleton: 195.4 (C-1), 177.9 (C-5), 162.3 (C-3), 96.3 (C-4), 55.1 (C-2), 41.9 (C-6), 28.9 (C-7), 24.8 and 18.9 (C-3' and C-5'), 22.9 and 21.8 (C-8a and C-8b); Sn-Ph skeleton ($^nJ(^{13}\text{C}-^{119/117}\text{Sn}, \text{ Hz})$): 137.9 (662/634) (C-1*), 136.9 (48) (C-2*), 130.3 (14) (C-4*), 129.0 (64) (C-3*), ppm. $^{119}\text{Sn-NMR}$ (CDCl_3): -103.7 ppm.

Synthesis of $[\text{Ph}_3\text{SnL}^2\text{H}]_n$ (**2**)

An identical method to that used for the preparation of **1** was followed using Ph_3SnCl and L^2HK . Yellow crystals of

Fig. 2 Three repeats of the crystallographically and chemically unique unit in the polymeric $[\text{Ph}_3\text{SnL}^3\text{H}]_n$ chain structure of **3** (50% probability ellipsoids). The H-atoms and one of the disordered conformations have been omitted for clarity



compound 2 were obtained from ethanol in 79% yield. M.p.: 153–55°C. Anal. Calc. for $C_{31}H_{31}NO_3Sn$: Theory: C, 63.73; H, 5.35; N, 2.40%. Found: C, 63.65; H, 5.30; N, 2.45%. IR absorptions (cm^{-1}): 1,646 $\nu(OCO)_{asym}$, 1,546 $\nu(C=N)$, 1,288 $\nu(Ph(CO))$. 1H -NMR ($CDCl_3$): Ligand skeleton: 13.3 (brs, 1H, OH), 8.32 (s, 1H, H-3'), 7.31 (t, 1H, H-7), 7.20 (d, 1H, H-9), 6.97 (d, 1H, H-6), 6.86 (t, 1H, H-8), 4.16 (d (3.3 Hz); $^3J(^{119/117}Sn-^1H=14\text{ Hz})$), 1H, H-2), 1.88 (s, 2H, H-10), 1.58 (s, 1H, H-11), 0.90 and 0.86 (d (7 Hz), 6H, H-12a and H-12b); Sn-Ph skeleton: 7.71 (m, 6H, H-2*), 7.45 (m, 9H, H-3* and H-4*), ppm. ^{13}C -NMR ($CDCl_3$): Ligand skeleton: 177.6 (C-1), 165.8 (C-5), 161.4 (C-3), 132.5 (C-7), 131.6 (C-9), 118.8 (C-4), 118.5 (C-8), 117.2 (C-6), 69.7 (C-2), 42.9 (C-10), 24.7 (C-11), 23.1 and 21.6 (C-12a and C-12b); Sn-Ph skeleton ($^nJ(^{13}C-^{119/117}Sn, \text{ Hz})$): 137.9 (654/627) (C-1*), 136.9 (48) (C-2*), 130.3 (16) (C-4*), 129.0 (63) (C-3*), ppm. ^{119}Sn -NMR ($CDCl_3$): -100.2 ppm.

Synthesis of $[Ph_3SnL^3]_n$ (3)

A mixture of Ph_3SnCl (0.80g, 2.07 mmol) and L^3HK (0.60g, 2.08 mmol) was refluxed in benzene (35 ml) for 6 h. The bright yellow solution was filtered and the filtrate was evaporated to a minimum and precipitated with hexane to give the crude product. The precipitate was filtered, dried in vacuo and upon recrystallization from ethanol furnished light fluorescent yellow crystals of 3 in 86% (1.07 g) yield. M.p.: 158–60°C. Anal. Calc. for $C_{32}H_{33}NO_3Sn$: Theory: C, 64.24; H, 5.56; N, 2.34%. Found: C, 63.89; H, 5.46; N, 2.21%. IR absorptions (cm^{-1}): 1,653 $\nu(OCO)_{asym}$, 1,613 $\nu(C=N)$, 1,261 $\nu(Ph(CO))$. 1H -NMR ($CDCl_3$): Ligand skeleton: 16.1 (brs, 1H, OH), 7.41 (m (overlapped with Sn-Ph H-3* and H-4*), 1H, H-9), 7.31 (t, 1H, H-7), 6.97 (d, 1H, H-6), 6.78 (t, 1H, H-8), 4.53 (d (3.3 Hz); $^3J(^{119/117}Sn-^1H=14\text{ Hz})$), 1H, H-2), 2.64 (s, 2H, H-10), 2.22 (s, 1H, H-3'), 1.93 (s, 1H, H-11), 0.92 and 0.85 (d (7 Hz), 6H, H-12a and H-12b); Sn-Ph skeleton: 7.65 (m, 6H, H-2*), 7.41 (m, 9H, H-3* and H-4*), ppm. ^{13}C -NMR ($CDCl_3$): Ligand skeleton: 177.5 (C-1), 172.3 (C-5), 163.8 (C-3), 132.6 (C-7), 128.3 (C-9), 118.9 (C-4), 118.8 (C-8), 117.1 (C-6), 60.8 (C-2), 43.1 (C-10), 25.0 (C-11), 23.0 and 21.9 (C-12a and C-12b), 14.9 (C-3'); Sn-Ph skeleton ($^nJ(^{13}C-^{119/117}Sn, \text{ Hz})$): 137.9 (654/627) (C-1*), 136.7 (50) (C-2*), 129.9 (16) (C-4*), 128.8 (64) (C-3*), ppm. ^{119}Sn -NMR ($CDCl_3$): -83.1 ppm.

Synthesis of $[Ph_2SnL^2H]_n$ (4)

Ph_2SnCl_2 (0.50 g, 1.42 mmol) in hot anhydrous benzene (45 ml) was added drop-wise to L^2HK (0.39 g, 1.42 mmol) suspended in anhydrous benzene (30 ml) with continuous stirring. The reaction mixture was refluxed for 1 h, then

triethylamine (0.15 ml, 1.42 mmol) was added and refluxing was continued for an additional 5 h. The reaction mixture was cooled to room temperature and filtered to remove $Et_3N.HCl$. The filtrate was collected; volatiles were removed in vacuo. The dried residue was washed thoroughly with hexane and then extracted into warm benzene (25 ml) and filtered. The filtrate was concentrated, precipitated with hexane, filtered and the light yellow residue obtained was dried in vacuo. The crude product was then re-crystallized from ethanol which afforded a lemon yellow microcrystalline product of 4 in 76.7% (0.69 g) yield. M.p.: 191–193°C (207–208°C [58]). Anal. Calc. for $C_{25}H_{25}NO_3Sn$: Theory: C, 59.32; H, 4.98; N, 2.77. Found: C, 59.29; H, 4.90; N, 2.84 %. IR absorptions (cm^{-1}): 1,686 $\nu(OCO)_{asym}$, 1,619 $\nu(C=N)$, 1,321 $\nu(Ph(CO))$. 1H -NMR ($CDCl_3$): Ligand skeleton: 8.22 (s, ($^3J(^{119/117}Sn-^1H=57\text{ Hz})$), 1H, H-3'), 7.55 (t, 1H, H-7), 7.19 (d, 1H, H-9), 7.15 (d, 1H, H-6), 6.81 (t, 1H, H-8), 4.17 (d (3.0 Hz); ($^3J(^{119/117}Sn-^1H=14\text{ Hz})$), 1H, H-2), 1.81 and 1.52 (m, 2H, H-10), 1.63 (m, 1H, H-11), 0.90 and 0.81 (d (7 Hz), 6H, H-12a and H-12b); Sn-Ph skeleton: 7.98 and 7.82 (m, 4H, H-2*), 7.47 and 7.36 (m, 6H, H-3* and H-4*), ppm. ^{13}C -NMR ($CDCl_3$): Ligand skeleton: 173.7 (C-1), 171.6 (C-5), 169.2 (C-3), 138.0 (C-7), , 135.5 (C-9), 122.8 (C-8), 117.8 (C-6), 117.1 (C-4), 67.6 (C-2), 44.7 (C-10), 23.7 (C-11), 22.7 and 21.8 (C-12a and C-12b); Sn-Ph skeleton ($^nJ(^{13}C-^{119/117}Sn, \text{ Hz})$): 137.5 (994) and 137.4 (962) (C-1*), 136.5 (57) and 136.2 (57) (C-2*), 130.7 (98) and 130.6 (78) (C-4*), 128.8 (17) and 128.7 (17) (C-3*), ppm. ^{119}Sn -NMR ($CDCl_3$): -340.5 ppm.

Experimental protocol and cytotoxicity tests

The experiment was started on day 0. On day 0, 10,000 cells per well were seeded into 96-wells flat-bottomed micro-titer plates (falcon 3072, DB). The plates were pre-incubated overnight at 37°C, 5% CO_2 to allow the cells to adhere to the bottom. On day 1, a three-fold dilution sequence of ten steps was made in full medium, starting with the 250,000 ng/ml stock solution. Every dilution was used in quadruplicate by adding 200 μ l to a column of four wells. This procedure results in the highest concentration of 625,000 ng/ml being present in column 12. Column 2 was used for the blank. After incubation of 3 days, the plates were washed with PBS twice. Fluorescein diacetate (FDA) stock solution was diluted to 2 μ g/ml with PBS and 200 μ l of this solution was added to each of the control, experimental and blank wells. The plates were incubated for 30 min at 37°C and the fluorescence generated from each well was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm using an automated microplate reader (Labsystems Multiskan MS). The data were used for construction of concentration-

response curves and determination of the ID_{50} values by use of Deltasoft 3 software.

The variability of the in vitro cytotoxicity test depends on the cell lines used and the serum applied. With the same batch of cell lines and the same batch of serum the inter-experimental CV (coefficient of variation) is 1–11% depending on the cell line and the intra-experimental CV is 2–4%. These values may be higher with other batches of cell lines and/or serum.

Results and discussion

Synthesis and spectroscopy

Potassium salts L^1HK - L^3HK were prepared by reacting acetylacetone, 2-hydroxybenzaldehyde or 2'-hydroxyacetophenone with potassium *l*-leucinate in methanol. Triphenyltin(IV) compounds viz., $[Ph_3SnL^1H]_n$ (1), $[Ph_3SnL^2H]_n$ (2) and $[Ph_3SnL^3H]_n$ (3) could easily be prepared by reacting potassium salts with Ph_3SnCl either in refluxing methanol or benzene in greater than 76% yields. On the other hand, $[Ph_2SnL^2H]_n$ (4) was prepared by reacting the appropriate potassium salt with Ph_2SnCl_2 in benzene in the presence of Et_3N as a proton abstractor, a method similar to that reported earlier [59]. The synthesis of these compounds is shown in Scheme 2. The compounds are shiny colourless to yellow solids. They are stable in air and soluble in all common organic solvents.

The IR spectra displayed a strong sharp band at around $1,650\text{ cm}^{-1}$ for triphenyltin(IV) complexes (1–3) and at around $1,685\text{ cm}^{-1}$ for diphenyltin(IV) complex (4) which has been assigned to the carboxylate antisymmetric $[\nu_{\text{asym}}(\text{OCO})]$ stretching vibration, in accord with our earlier reports [39, 42, 43, 47, 49]. The assignment of the symmetric $[\nu_{\text{sym}}(\text{OCO})]$ stretching vibration band could not be made owing to the complex pattern of the spectra. The 1H - and ^{13}C - NMR signals were assigned by the use of homonuclear correlated spectroscopy (COSY), heteronuclear single-quantum correlation (HSQC), heteronuclear multiple-bond connectivities (HMBC) and distortionless enhancement by polarization transfer (DEPT) experiments. The 1H and ^{13}C chemical shift assignment (see Experimental) of the phenyltin moiety is straightforward from the multiplicity patterns, resonance intensities and also by comparing their $^nJ(^{13}C-^{119/117}Sn)$ values. The 1H -NMR integration values were completely consistent with the formulation of the products. The ^{13}C -NMR spectra of the ligand and Sn-Ph skeletons displayed the expected carbon signals in triphenyltin(IV) complexes 1–3. The diphenyltin(IV) complex (4) deserves specific mention. The spin-spin coupling of 57 Hz between the azomethine proton and the tin nucleus, $^3J(^{119/117}Sn=N=C^1H)$ has been

detected. Such coupling has previously been reported for the complexes where tin nuclei are located in a *trans*-position to the azomethine proton [60], confirming the presence of nitrogen-tin coordination. In addition, complex 4 ($Ph_2Sn(2-OC_6H_4C(H)=NCHCH_2CH(CH_3)_2COO)$) displayed two sets of 1H and ^{13}C NMR signals from the Sn-Ph groups (see experimental) since the italicised proton in the complex is enantiotropic and causes the phenyl groups bound to the tin, and the iBu group to be diastereotopic [61], and thereby the two phenyl groups (Sn- Ph_2) experience different environments on the NMR time scale. These observations were noted earlier for diorganotin(IV) complexes of 2- $\{[(E)-1-(2\text{-oxyphenyl})\text{alkylidene}]\text{amino}\}$ phenylpropionate [49]. The triphenyltin(IV) complexes (1–3) in $CDCl_3$ exhibit a single sharp ^{119}Sn resonance in the range -83 to -103 ppm, suggesting that the Sn-atoms in the complexes have the same four-coordinate environment [42, 47, 49, 62, 63]. This is also reflected in $^1J(^{13}C-^{119/117}Sn)$ coupling constants (see experimental) and the values are unambiguously characteristic for four-coordinate tin atoms [63]. These results demonstrate that the polymeric structure with five-coordinate tin atoms found in the solid state is lost upon dissolution (see below for the crystal structure discussion). On the other hand, the X-ray crystal structure of complex 4 demonstrated a pseudo-trigonal bipyramid geometry around the tin atom [58]. The chemical shift data for this complex in $CDCl_3$ displayed a ^{119}Sn chemical shift at -340 ppm, which closely matches the shifts reported for diphenyltin(IV) amino acetate [39, 41], which has five-coordinate Sn-atoms in solution. This shift testifies that the solid state structure of 4, revealed in the crystal structure, is retained in solution.

Crystal structures

The crystal structures of compounds 1 and 3 reveal that the Sn-coordination complex in each case has the basic type X polymeric chain structural motif described in Scheme 1. A preliminary investigation of the crystal structure of 2 revealed the same motif, but this structure is not described in any further detail here (see the “Experimental” for details). Views of the molecular structures of complexes 1 and 3 are shown in Figs. 1 and 2 (refer to Scheme 2 for line diagrams), while selected geometric parameters are collected in Table 2.

The following specific details are given for the structure of 1, but the comments apply equally well to the structures of 2 and 3. It is apparent that varying the organic moiety in the Schiff base ligands has an insignificant influence on the overall structure and the coordination geometry of the Sn-atoms. Complex 1 has a polymeric chain structure in which a single carboxylate ligand bridges adjacent Sn-centres via

Table 2 Selected bond lengths (Å) and angles (°) for compounds (1 and 3)

1		3	
Sn(1)–O(1)	2.151(2)	Sn–O(1)	2.197(4)
Sn(1)–O(2)	3.386(3)	Sn–O(2)	3.537(5)
Sn(1)–C(11)	2.136(4)	Sn–C(14)	2.124(5)
Sn(1)–C(17)	2.132(4)	Sn–C(20)	2.118(5)
Sn(1)–C(23a)	2.132(4)	Sn–C(26a)	2.135(5)
Sn(1)–C(23b)	2.129(5)	Sn–C(26b)	2.143(5)
Sn(1)–O(3')	2.352(2)	Sn–O(3')	2.242(4)
O(1)–Sn(1)–C(11)	97.3(1)	O(1)–Sn–C(14)	94.4(2)
O(1)–Sn(1)–C(17)	95.6(1)	O(1)–Sn–C(20)	92.6(2)
O(1)–Sn(1)–C(23a)	85.3(4)	O(1)–Sn–C(26a)	90.5(6)
O(1)–Sn(1)–C(23b)	90.7(6)	O(1)–Sn–C(26b)	80.4(4)
C(11)–Sn(1)–C(17)	122.2(1)	C(14)–Sn–C(20)	120.2(2)
C(17)–Sn(1)–C(23a)	119.8(4)	C(20)–Sn–C(26a)	109.7(5)
C(17)–Sn(1)–C(23b)	111.8(6)	C(20)–Sn–C(26b)	120.2(5)
C(23a)–Sn(1)–C(11)	117.2(5)	C(26a)–Sn–C(14)	129.5(4)
C(23b)–Sn(1)–C(11)	124.0(7)	C(26b)–Sn–C(14)	119.5(4)
O(3')–Sn(1)–C(11)	87.5(1)	O(3')–Sn–C(14)	89.1(2)
O(3')–Sn(1)–C(17)	87.5(1)	O(3')–Sn–C(20)	93.0(2)
O(3')–Sn(1)–C(23a)	86.4(4)	O(3')–Sn–C(26a)	80.7(6)
O(3')–Sn(1)–C(23b)	81.0(6)	O(3')–Sn–C(26b)	90.5(4)
O(1)–Sn(1)–O(3')	171.7(1)	O(1)–Sn–O(3')	170.8(1)
C(1)–O(1)–Sn(1)	125.5(2)	C(1)–O(1)–Sn	130.9(3)
C(9')–O(3')–Sn(1)	134.9(2)	C(13')–O(3')–Sn	130.2(3)

Primed atoms refer to atoms from the next symmetrically-related ligand in the polymeric chain (symmetry code for 1: $1-x, -\frac{1}{2}+y, \frac{1}{2}-z$; for 3: $2-x, \frac{1}{2}+y, 1-z$)

its carboxylate and oxide O-atoms. The asymmetric unit contains one of the chemical repeat units of the polymeric Sn-compound. Compounds 1 and 3 in the crystal are enantiomerically pure and the absolute configuration of the molecules has been determined independently by the diffraction experiments. The stereogenic centre at C(2) of the molecules 1 and 3 has the *S*-configuration expected of *L*-leucine.

The primary coordination sphere of the Sn-atom is trigonal bipyramidal with the phenyl ligands in the equatorial plane and the axial positions occupied by one O-atom from the carboxylate group of one carboxylate ligand and the oxide O-atom of the next carboxylate ligand in the chain. This yields a *trans*-R₃SnO₂ geometry. The second O-atom of the carboxylate group is not involved in the primary coordination sphere of the Sn-atom, but lies 3.386(3) Å (3.537(5) Å in 3) from the Sn-atom. Polymeric chain structures involving triphenyltin(IV) and the Schiff bases of amino acids with a similar mode of coordination and geometry about the Sn-atom have been observed in triphenyltin 2-{[(*E*)-1-(2-hydroxyphenyl)methylidene]amino}acetate [42], 2-{[(*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}phenylpropionate [49], 2-{[(*E*)-1-(2-hydroxyphenyl) methylidene]amino}phe-

nylpropionate [49] and 2-{[(*E*)-1-(2-hydroxyphenyl)ethylidene]amino}- phenylpropionate [49]. Among all of these structures, the greatest variation in the coordination geometry is in the Sn–O(oxide) bond length, which ranges from 2.242 (2) Å in 3 to 2.539(2) Å in 2-{[(*E*)-1-(2-hydroxyphenyl) methylidene]amino}phenylpropionate [49]. The formal hydroxy group in the carboxylate ligand of 1 and 3 has lost its H-atom, so is negatively charged. Instead the imine N-atom is protonated, thus leading to a zwitterionic ligand. This N⁺–H group forms a strong intramolecular hydrogen bond with the oxide O atom (Table 3). One of the phenyl ligands and the *iso*-propyl group in 1 are disordered over two orientations while one of the phenyl ligands is disordered over two almost equally occupied orientations in 3.

The polymeric structures of the triphenyltin(IV) complexes 1–3 is not repeated in the crystal structure of diphenyltin(IV) complex 4, which was described by Wang et al. [58]. In 4, a mononuclear discrete molecule, the arrangement of the donor set about the Sn-atom is distorted trigonal bipyramidal with one carboxylate and the oxide oxygen atoms from the tridentate carboxylate ligand occupying the axial positions, while the imine nitrogen atom from the carboxylate ligand and two phenyl groups are in equatorial positions (refer to Scheme 2).

Cytotoxicity studies

The in vitro cytotoxicity test of organotin(IV) compounds 1–4 was performed using the SRB test for the estimation of cell viability. The cell lines WIDR, M19 MEL, A498, IGROV and H226 belong to the currently used anticancer screening panel of the NCI, USA [64]. The MCF7 cell line is estrogen receptor (ER)+/ progesterone receptor (PgR)+ and the cell line EVSA-T is (ER)–/(PgR)–. Prior to the experiments, a mycoplasma test was carried out on all cell lines and found to be negative. All cell lines were maintained in a continuous logarithmic culture in RPMI 1640 medium with Hepes and phenol red. The medium was supplemented with 10% FCS, penicillin 100 µg/ml and streptomycin 100 µg/ml. The cells were mildly trypsinized for passage and for use in the experiments. RPMI and FCS were obtained from Life technologies (Paisley, Scotland). SRB, DMSO, Penicillin and streptomycin were obtained from Sigma (St. Louis MO, USA), TCA and acetic acid from Baker BV (Deventer, NL) and PBS from NPBI BV

Table 3 Hydrogen bonding geometry (Å, °) for compounds 1 and 3

	D–H⋯A	D–H	H⋯A	D⋯A	D–H⋯A
1	N(1)–H(1)⋯O(3)	0.86(4)	2.00(3)	2.668(4)	134(3)
3	N(1)–H(1)⋯O(3)	0.88	1.80	2.546(5)	141

(Emmer-Compascuum, NL). The test compounds 1–4 and reference compounds were dissolved to a concentration of 250,000 ng/ml in full medium, by 20 fold dilution of a stock solution which contained 1 mg of compounds 1–4/200 μ l. All the four compounds were dissolved in DMSO. Cytotoxicity was estimated by the microculture sulforhodamine B (SRB) test [65].

The results of the in vitro cytotoxicity tests performed with triphenyltin(IV) compounds, (1–3) and diphenyltin(IV) compound (4) are summarized in Table 4 and the screening results are compared with the results from other related triphenyltin(IV)- and diorganotin(IV)- compounds with respect to the standard drugs that are in current clinical use as antitumour agents. Recently, we have reported in vitro cytotoxic results on triphenyltin(IV) compounds 5–7

where the ligand is a Schiff base derived from phenylalanine [49]. There are also reports on di-*n*-butyltin(IV) and diphenyltin(IV) compounds with Schiff bases derived from glycine, β -alanine, and *l*-leucine and these were also investigated for in vitro cytotoxic potential (see Table 4 for details) [66]. The di-*n*-butyltin(IV) compounds of cognate systems were also investigated for antitumour activity against the NCI panel of 60 cell lines [67]. The results indicated that three of the di-*n*-butyltin(IV) compounds, viz., $^n\text{Bu}_2\text{Sn}(2\text{-OC}_6\text{H}_4\text{C(H)=NCH}_2\text{COO})$, $^n\text{Bu}_2\text{Sn}(2\text{-OC}_6\text{H}_4\text{C(CH}_3\text{)=NCH}_2\text{COO})$ and $^n\text{Bu}_2\text{Sn}(2\text{-OC}_6\text{H}_4\text{C(H)=NCHCH(CH}_3\text{)}_2\text{COO})$ exhibited very high cytotoxic effects on the NCI-522 (non-small cell lung cancer) cell line. The fourth compound, $^n\text{Bu}_2\text{Sn}(2\text{-OC}_{10}\text{H}_6\text{C(H)=NCH}_2\text{COO})$ exhibited highest cytotoxic activity on the cell line RXF-

Table 4 In vitro ID₅₀ values (ng/ml) of test compounds 1–4 and some reported organotin(IV) compounds compared with some standard drugs used in cell viability tests against seven human tumour cell lines

Test compound ^a	Cell lines						
	A498	EVSA-T	H226	IGROV	M19 MEL	MCF-7	WIDR
[Ph ₃ SnL ¹ H] _n (1)	96	35	56	90	42	36	33
[Ph ₃ SnL ² H] _n (2)	104	49	111	99	75	76	42
[Ph ₃ SnL ³ H] _n (3)	39	31	38	46	36	34	31
Ph ₂ SnL ² (4)	987	278	849	199	409	452	405
[Ph ₃ SnL ⁴ H] _n . <i>n</i> CCl ₄ (5) ^b [49]	105	81	105	101	102	111	106
[Ph ₃ SnL ⁵ H] _n (6) ^b [49]	120	100	115	105	130	115	110
[Ph ₃ SnL ⁶ H] _n (7) ^b [49]	113	96	108	106	112	110	109
{[ⁿ Bu ₂ Sn(2-OHC ₆ H ₄ C(CH ₃)=N(CH ₂) ₂ COO)] ₂ O} ₂ [47]	376	34	237	174	225	147	895
DOX	90	8	199	60	16	10	11
CDDP	2253	422	3269	169	558	699	967
5-FU	143	475	340	297	442	750	225
MTX	37	5	2287	7	23	18	<3.2
ETO	1314	317	3934	580	505	2594	150
TAX	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2	<3.2
$^n\text{Bu}_2\text{Sn}(2\text{-OC}_{10}\text{H}_6\text{C(H)=NCH}_2\text{COO})$ [66]	170	35	190	75	90	75	480
$^n\text{Bu}_2\text{Sn}(2\text{-OC}_{10}\text{H}_6\text{C(H)=N(CH}_2\text{)}_2\text{COO})$ [66]	62	17	160	27	71	20	114
$^n\text{Bu}_2\text{Sn}(2\text{-OC(CH}_3\text{)C(H)C(CH}_3\text{)=NCHCH}_2\text{CH(CH}_3\text{)}_2\text{COO})$ [66]	130	120	200	130	70	60	420
Ph ₂ Sn(2-OC ₁₀ H ₆ C(H)=NCH ₂ COO) [66]	230	70	350	120	530	170	490
Ph ₂ Sn(2-OC ₁₀ H ₆ C(H)=N(CH ₂) ₂ COO) [66]	690	150	1100	480	620	600	1750
DOX	55	13	180	150	21	25	18
CDDP	1200	920	3160	230	780	1400	1550
5-FU	340	720	5300	850	310	350	440
MTX	16	26	70	20	18	15	7
Carboplatin	18,000	4,500	25,000	2,400	5,500	10,500	3,500
Ph ₃ SnR ₁ ^c [27]	42	<3	39	19	42	17	17
Ph ₃ SnR ₂ ^c [27]	65	<3	61	18	51	16	19
Ph ₃ SnR ₃ ^c [27]	<2	<2	<2	<2	<2	2.9	<2
CDDP	2,253	422	3,269	169	558	699	967
DOX	90	8	199	60	16	10	11

DOX doxorubicin, CDDP cisplatin, 5-FU 5-fluorouracil, MTX methotrexate, ETO etoposide, TAX paclitaxel

^a Standard drug reference values are cited immediately after the test compounds under identical conditions

^b Reported triphenyltin(IV) compounds (5–7) have been included for comparison; 49: LH is a carboxylate residue where L⁴H, 2-[(2Z)-(3-hydroxy-1-methyl-2-butenylidene)]amino}phenylpropionate; L⁵H, 2-[(E)-1-(2-hydroxyphenyl)methylidene]amino}phenylpropionate; L⁶H, 2-[(E)-1-(2-hydroxyphenyl)ethylidene]amino}phenylpropionate

^c Reported triphenyltin(IV) compounds, R is a carboxylate residue where R₁=-terebate, R₂=-steroidcarboxylate, R₃=-benzocrown carboxylate

631L (renal cancer). In general, a low to moderate cellular response was observed for all the di-*n*-butyltin(IV) compounds, with at least one cell line in each subpanel of cells exhibiting a very low growth inhibition response. The results also indicated that the compounds did not exhibit any significant subpanel activity and suggested that the di-*n*-butyltin(IV) compounds were not active in all the cell lines contained in any subpanel. The di-*n*-butyltin(IV) compound of composition $\{[{}^n\text{Bu}_2\text{Sn}(2\text{-OHC}_6\text{H}_4\text{C}(\text{CH}_3)=\text{N}(\text{CH}_2)_2\text{COO})_2\text{O}]\}_2$ (Table 4) also exhibited good activity especially when compared with CCDP [47]. Another interesting mixed organotin(IV) binuclear compound $\text{Ph}_3\text{Sn}(\text{HL})\cdot\text{Ph}_2\text{SnL}$ ($\text{L}=2\text{-O-3,5-Br}_2\text{C}_6\text{H}_2\text{C}(\text{H})=\text{NCHCH}(\text{CH}_3)_2\text{COO}$) also displayed good in vitro cytotoxicity against three human tumour cell lines, i.e. HeLa (cervix tumour cell), CoLo 205 (colon carcinoma cell) and MCF-7 (mammary tumour cell) when compared with CCDP [68, 69].

The triphenyltin(IV) compounds of the present investigation (1–3, Table 4) showed ID_{50} values in the range 33–96, 42–104 and 31–38 ng/ml, respectively, whereas diphenyltin(IV) compound 4 produced an ID_{50} value in the range 199–987 ng/ml, across seven human tumour cell lines. Triphenyltin(IV) compound 3 is the most promising candidate in this study and the most cytotoxic organotin(IV) complex so far with a Schiff base bearing amino acetate skeleton. Compound 3 is around 25 fold more active in magnitude in terms of the ID_{50} value at least against the A498 and H226 cell lines and is found to be almost as cytotoxic as MTX and found to be much better particularly for the H226 cell line. Under identical conditions, compound 3 is far superior to CCDP across a panel of cell lines and the activity is more pronounced for the A498 (57 fold) and H226 (86 fold) cell lines. The increase in the cytotoxicity of triphenyltin(IV) compounds 1–3 across the cell lines is likely to be because of the non-involvement of the nitrogen atom in the complexation with the tin atom (see Scheme 2 and Fig. 2), which allows easy dissociation of the complex so that it can subsequently bind to DNA. This corroborates the fact that diorganotin(IV) complexes show lower activity when the nitrogen atom coordinates to the tin atom [67]. A typical mode of bonding of diorganotin(IV) complexes is shown in Scheme 2 taking complex 4 as an example. However, the variations in in vitro cytotoxicity of the triphenyltin(IV) complexes may be due to different kinetic and mechanistic behaviour [49]. Conversely, the possibility of organotin compounds interacting with DNA at the level of the phosphate groups cannot be completely ruled out [70]. In conclusion, the present study describes new structures with improved in vitro anti-tumour activity, which is of added value in determining the structure activity relationship in the area of organotin(IV) chemistry with possible future clinical application.

Conclusions and outlook

The manuscript reports the preparation and crystal structures of some novel triphenyltin(IV) complexes which may find applications in cancer chemotherapy. The most promising triphenyltin(IV) compound 3 shows the highest cytotoxicity so far among the organotin(IV) compounds containing Schiff base amino acetate systems when tested in vitro across seven human tumour cell lines, indicating its high potential as an anti-cancer drug. It is intended to employ these compounds for testing in animal models and additionally to search for differently substituted Schiff base amino acetate skeletons in order to improve the solubility. Further work in this area is underway.

Acknowledgements The financial support of the Department of Science & Technology, New Delhi, India (Grant No.SR/S1/IC-03/2005,TSBB) and the University Grants Commission, New Delhi, India through SAP-DSA, Phase-III, are gratefully acknowledged. The in vitro cytotoxicity experiments were carried out by Ms. P. F. van Cuijk in the Laboratory of Translational Pharmacology, Department of Medical Oncology, Erasmus Medical Center, Rotterdam, The Netherlands, under the supervision of Dr. E. A. C. Wiemer and Prof. Dr. G. Stoter.

Appendix

Supplementary Material

CCDC-696775 and CCDC-696776 contain the supplementary crystallographic data for complexes 1 and 3, respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

References

- Schwartzmann G, Ratain MJ, Cragg GM, Wong JE, Saijo N, Parkinson DR, Fujiwara Y, Pazdur R, Newman DJ, Dagher R, Di Leone L (2002) Anticancer drug discovery and development throughout the world. *J Clin Oncol* 20(Suppl 15):47S–59S
- Neidle S, Thurston DE (2005) Chemical approaches to the discovery and development of cancer therapies. *Nat Rev Cancer* 5:285–296. doi:10.1038/nrc1587
- Narayanan VL, Nasr M, Paul KD (1990) In: Gielen M (ed) Tin-based antitumor drugs. Springer, Berlin, pp 201–217
- Crowe AJ (1988) In: Gielen M (ed) Metal based antitumour drugs, vol. 1. Freund, London, pp 103–149
- Gielen M, Lelieveld P, de Vos D, Willem R (1992) In: Gielen M (ed) Metal based antitumour drugs, vol. 2. Freund, Tel Aviv, pp 29–54
- Pellerito C, Agati PD, Fiore T, Mansueto C, Mansueto V, Stocco G, Nagy L, Pellerito L (2005) Synthesis, structural investigations on organotin(IV) chlorin-*e*₆ complexes, their effect on sea urchin embryonic development and induced apoptosis. *J Inorg Biochem* 99:1294–1305. doi:10.1016/j.jinorgbio.2005.03.002

7. Cima F, Ballarin L (1999) TBT-induced apoptosis in tunicate haemocytes. *Appl Organomet Chem* 13:697–703. doi:10.1002/(SICI)1099-0739(199910)13:10<697::AID-AOC916>3.0.CO;2
8. Shuaibu MN, Kanbara H, Yanagi T, Ichinose A, Ameh DA, Bonire JJ, Nok AJ (2003) In vitro trypanocidal activity of dibutyltin dichloride and its fatty acid derivatives. *Jpn Parasitol Res* 91:5–11. doi:10.1007/s00436-003-0861-2
9. Jan C-R, Jiann B-P, Lu Y-C, Chang H-T, Su W, Chen W-c, Yu C-C, Huang J-K (2002) Effect of the organotin compound triethyltin on Ca^{2+} handling in human prostate cancer cells. *Life Sci* 70:1337–1345. doi:10.1016/S0024-3205(01)01500-4
10. Samuel MP, de Vos D, Raveendra D, Sarma JARP, Roy S (2002) 3-D QSAR studies on new dibenzyltin(IV) anticancer agents by comparative molecular field analysis (CoMFA). *Bioorg Med Chem Lett* 12:61–64. doi:10.1016/S0960-894X(01)00684-9
11. Carraher CE Jr, Battin A, Shahi KR, Roner MR (2007) Synthesis, structural characterization, and initial evaluation as anticancer Drugs of dibutyltin polyamines derived from various 4,6-diaminopyrimidines. *J Inorg Organomet Polym* 17:631–639. doi:10.1007/s10904-007-9159-7
12. Barot G, Shahi KR, Roner MR, Carraher CE Jr (2007) Synthesis, structural characterization, and ability to Inhibit cancer growth of a series of organotin poly(ethylene glycols). *J Inorg Organomet Polym* 17:595–603. doi:10.1007/s10904-007-9158-8
13. Roner M, Carraher C, Sabir T, Shahi K, Roehr J, Bassett K (2006) Anticancer and antiviral activities of organotin polyether amines derived from the antiviral acyclovir. *Polym Mater Sci Eng* 95:525–527
14. Carraher C, Ashida Y, Battin G (2006) Synthesis of organotin polyethers containing diethylstilbestrol. *Polym Mater Sci Eng* 95:556–558
15. Carraher C, Sabir T, Roner M, Shahi K, Bleicher R, Roehr J, Bassett K (2006) Synthesis of organotin polyamine ethers containing acyclovir and their preliminary anticancer and antiviral activity. *J Inorg Organomet Polym* 16:249–257. doi:10.1007/s10904-006-9050-y
16. Roner M, Carraher C, Roehr J, Bassett K (2006) Antiviral and anticancer activity of organotin polymers and reactants derived from norfloxacin and ampicillin. *J Polym Mater* 23:153–159
17. Carraher C, Siegmund-Louda D (2004) Macromolecules containing metal and metal-like Elements, vol 3. Biomedical applications. Wiley, Hoboken, NJ
18. Doucette R, Siegmund-Louda D, Carraher C, Cardoso A (2004) Inhibition of Balb 3T3 cells as a function of metal for kinetin containing polymers. *Polym Mater Sci Eng* 91:564–566
19. Blower PJ (2004) Inorganic pharmaceuticals. *Annu Rep Prog Chem Sect A* 100:633–658. doi:10.1039/b312109g
20. Gielen M (2003) An overview of forty years organotin chemistry developed at the Free Universities of Brussels ULB and VUB. *J Braz Chem Soc* 14:870–877. doi:10.1590/S0103-50532003000600003
21. Höti N, Ma J, Tabassum S, Wang Y, Wu M (2003) Triphenyl tin benzimidazolethiol, a novel antitumor agent, induces mitochondrial-mediated apoptosis in human cervical cancer cells *via* suppression of HPV-18 encoded E6. *J Biochem* 134:521–528. doi:10.1093/jb/mvg169
22. Xanthopoulou MN, Hadjikakou SK, Hadjiliadis N, Schürmann M, Jurkschat K, Michaelides A, Skoulaka S, Bakas T, Binolis J, Karkabounas S, Charalabopoulos K (2003) Synthesis, structural characterization and in vitro cytotoxicity of organotin(IV) derivatives of heterocyclic thioamides, 2-mercaptobenzothiazole, 5-chloro-2-mercaptobenzothiazole, 3-methyl-2-mercaptobenzothiazole and 2-mercaptosuccinic acid. *J Inorg Biochem* 96:425–434. doi:10.1016/S0162-0134(03)00178-8
23. Höti N, Zhu D-e, Song Z, Wu Z, Tabassum S, Wu M (2004) p53-dependent apoptotic mechanism of a new designer bimetallic compound tri-phenyl tin benzimidazolethiol copper chloride (TPT-CuCl₂): In vivo studies in Wistar rats as well as in vitro studies in human cervical cancer cells. *J Pharmacol Exp Ther* 311:22–33. doi:10.1124/jpet.104.069104
24. Chasapis CT, Hadjikakou SK, Garoufis A, Hadjiliadis N, Bakas T, Kubicki M, Ming Y (2004) Organotin(IV) derivatives of L-cysteine and their *in vitro* anti-tumor properties. *Bioinorg Chem Appl* 2:43–54. doi:10.1155/S1565363304000044
25. Maher T, Snyder J, Durham P, Gerasimchuk NN (2005) Proceedings of the 229th ACS National Meeting, San Diego, CA, United States, March 13–17, INOR-719 (abstracts)
26. Tabassum S, Pettinari C (2006) Chemical and biotechnological developments in organotin cancer chemotherapy. *J Organomet Chem* 691:1761–1766. doi:10.1016/j.jorganchem.2005.12.033
27. Gielen M, Tiekink ERT (eds) (2005) Metallotherapeutic drug and metal-based diagnostic agents: ⁵⁰Sn Tin compounds and their therapeutic potential. Wiley, Chichester, England, pp 421–439 (and references therein)
28. Blunden SJ, Evans CJ (1990) In: Hutziger O (ed.) *Anthropogenic compounds*. Springer, Heidelberg, pp 1–44
29. Evans CJ (1998) In: Smith PJ (eds.) *Chemistry of tin*. Blackie Academic, London, pp 442–479
30. Willem R, Bouhddid A, Mahieu B, Ghys L, Biesemans M, Tiekink ERT, de Vos D, Gielen M (1997) Synthesis, characterization and in vitro antitumor activity of triphenyl- and tri-n-butyltin benzoates, phenylacetates and cinnamates. *J Organomet Chem* 531:151–158. doi:10.1016/S0022-328X(96)06686-7
31. Gielen M, El Khoulfi A, Biesemans M, Bouhddid A, de Vos D, Mahieu B, Willem R (1994) Synthesis, characterization and high in vitro antitumor activity of novel triphenyltin carboxylates. *Metal-Based Drugs* 1:305–309. doi:10.1155/MBD.1994.305
32. Bouālam M, Gielen M, El Khoulfi A, de Vos D, Willem R (1993) Novel organo-tin compounds having anti-tumor activity and antitumor compositions. *Pharmachemie B.V., Eur Pat, Publ* 538 517, Appl. 91/202, 746.3-, 22.10.91. *Chem Abstr* 119:117548b
33. Gielen M, Willem R, Biesemans M, Bouālam M, El Khoulfi A, de Vos D (1992) Exceptionally high in vitro antitumor activity of substituted triphenyltin benzoates including salicylates against a human mammary tumour, MCF-7, and a colon carcinoma, WiDr. *Appl Organomet Chem* 6:287–291. doi:10.1002/aoc.590060307
34. Kemmer M, Gielen M, Biesemans M, de Vos D, Willem R (1998) Synthesis, characterization and in vitro antitumor activity of di-n-butyl, tri-n-butyl and triphenyltin 3,6-dioxahexanoates and 3,6,9-trioxadecanoates. *Metal-Based Drugs* 5:189–196. doi:10.1155/MBD.1998.189
35. Kemmer M, Ghys L, Gielen M, Biesemans M, Tiekink ERT, Willem R (1999) Synthesis and characterization of triphenyl-, tri-n-butyl and di-n-butyltin derivatives of 4-carboxybenzo-18-crown-6 and -15-crown-5. *J Organomet Chem* 582:195–203. doi:10.1016/S0022-328X(99)00035-2
36. Gielen M, Lelieveld P, de Vos D, Pan H, Willem R, Biesemans M, Fiebig HH (1992) In vitro effect of organotin-substituted steroids in human tumor cell lines. *Inorg Chim Acta* 196:115–117. doi:10.1016/S0020-1693(00)82967-9
37. Gielen M, Willem R, Dalil H, de Vos D, Kuiper CM, Peters GJ (1998) Toxicity profiles in vivo in mice and antitumor activity in tumour-bearing mice of di- and triorganotin compounds. *Metal-Based Drugs* 5:83–90. doi:10.1155/MBD.1998.83
38. Tiekink ERT (1994) The rich diversity in tin carboxylate structures. *Trends Organomet Chem* 1:71–116
39. Dakternieks D, Basu Baul TS, Dutta S, Tiekink ERT (1998) Synthesis, characterization, and X-ray structures of diphenyltin (IV) *N*-(2-hydroxyacetophenone)glycinate, its 1:1 adduct with triphenyltin(IV) chloride, and related systems. *Organometallics* 17:3058–3062. doi:10.1021/om9800290
40. Basu Baul TS, Dutta S, Tiekink ERT (1999) Crystal structure of diphenyltin(IV) *N*-(2-hydroxy-5-methylacetophenone)glycinate, C₂₃H₂₁NO₃Sn. *Z Kristallogr* 214:361–362

41. Basu Baul TS, Dutta S, Rivarola E, Choudhuri S (2001) Synthesis, characterization of diorganotin(IV) complexes of *N*-(2-hydroxyarylidene)aminoacetic acid and antitumour screening in vivo in Ehrlich ascites carcinoma cells. *Appl Organomet Chem* 15:947–953. doi:10.1002/aoc.245
42. Basu Baul TS, Dutta S, Rivarola E, Butcher R, Smith FE (2002) The synthesis and structural characterization of some triorganotin(IV) complexes of 2-[(*E*)-1-(2-hydroxyaryl)alkylidene]amino}acetic acid. Crystal and molecular structures of $\text{Ph}_3\text{Sn}(2\text{-OHC}_6\text{H}_4\text{C(H)=NCH}_2\text{COO})$ and $\text{Me}_3\text{Sn}(2\text{-OHC}_6\text{H}_4\text{C(CH}_3\text{)=NCH}_2\text{COO})$. *J Organomet Chem* 654:100–108. doi:10.1016/S0022-328X(02)01387-6
43. Basu Baul TS, Dutta S, Masharing C, Rivarola E, Englert U (2003) Organotin(IV) complexes of *N*-[(2*Z*)-3-hydroxy-1-methyl-2-butenylidene]glycine. *Heteroatom Chem* 14:149–154. doi:10.1002/hc.10116
44. Yin H, Wang Q, Xue S (2004) Synthesis and structural characterization of diorganotin(IV) esters of salicylidene-amino acids. *J Organomet Chem* 689:2480–2485. doi:10.1016/j.jorganchem.2004.05.004
45. Basu Baul TS, Masharing C, Willem R, Bieseman M, Holčapek M, Jirásko R, Linden A (2005) Self-assembly of diorganotin(IV) 2-[(*E*)-1-(2-oxyaryl)alkylidene]amino}acetates: An investigation of structures by X-ray diffraction, solution and solid state tin NMR, and electrospray ionisation MS. *J Organomet Chem* 690:3080–3094. doi:10.1016/j.jorganchem.2005.03.010
46. Linden A, Basu Baul TS, Masharing C (2005) Chloro{ μ -2-[(*E*)-1-(2-oxido-3-methylphenyl) ethylideneamino]acetato}pentaphenylditin(IV). *Acta Crystallogr E* 61:m557–m559
47. Basu Baul TS, Masharing C, Basu S, Rivarola E, Holčapek M, Jirásko R, Lyčka A, de Vos D, Linden A (2006) Synthesis, characterization, cytotoxic activity and crystal structures of tri- and di-organotin(IV) complexes constructed from the β -{[(*E*)-1-(2-hydroxyaryl)alkylidene]amino}propionate and β -{[(2*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}propionate skeletons. *J Organomet Chem* 691:952–965. doi:10.1016/j.jorganchem.2005.10.057
48. Basu Baul TS, Masharing C, Rivarola E, Smith FE, Butcher J (2006) Synthesis and characterization of tribenzyltin(IV) and dibenzyltin(IV) complexes of 2-[(2*Z*)-3-hydroxy-1-methyl-2-butenylidene]amino}acetic acid. Crystal structure of tribenzyl{2-[(2*Z*)-3-hydroxy-1-methyl-2-butenylidene]amino}acetate}tin(IV). *Struct Chem* 18:231–235. doi:10.1007/s11224-006-9085-2
49. Basu Baul TS, Masharing C, Ruisi G, Jirásko R, Holčapek M, de Vos D, Wolstenholme D, Linden A (2007) Self-assembly of extended schiff base amino acetate skeletons, 2-[(2*Z*)-(3-hydroxy-1-methyl-2-butenylidene)]amino}phenylpropionate and 2-[(*E*)-1-(2-hydroxyaryl)alkylidene]amino}phenylpropionate skeletons incorporating organotin(IV) moieties: Synthesis, spectroscopic characterization, crystal structures, and in vitro cytotoxic activity. *J Organomet Chem* 692:4849–4862. doi:10.1016/j.jorganchem.2007.06.061
50. Hoofst R (1999) KappaCCD Collect Software, Nonius BV, Delft, The Netherlands
51. Otwinowski Z, Minor W (1997) Processing of X-ray diffraction data collected in oscillation mode. In: Carter Jr CW, Sweet RM (eds) *Methods in Enzymology: Macromolecular Crystallography*, Part A. vol. 276. Academic, New York, pp 307–326
52. Blessing RH (1995) An empirical correction for absorption anisotropy. *Acta Crystallogr A* 51:33–38. doi:10.1107/S0108767394005726
53. Altomare A, Casciarano G, Giacovazzo C, Guagliardi A, Burla MC, Polidori G, Camalli M (1994) *SIR92* - a program for automatic solution of crystal structures by direct methods. *J Appl Cryst* 27:435. doi:10.1107/S002188989400021X
54. Beurskens PT, Admiraal G, Beurskens G, Bosman WP, Garcia-Granda S, Gould RO, Smits JMM, Smykalla C (1992) *PATY*: The DIRDIF program system, Technical report of the crystallography laboratory. University of Nijmegen, The Netherlands
55. Beurskens PT, Admiraal G, Beurskens G, Bosman WP, de Gelder R, Israel R, Smits JMM (1994) *DIRDIF94*: The DIRDIF program system, technical report of the crystallography laboratory. University of Nijmegen, The Netherlands
56. Flack HD, Bernardinelli G (1999) Absolute structure and absolute configuration. *Acta Crystallogr A* 55:908–915; Flack HD, Bernardinelli G (2000) Reporting and evaluating absolute-structure and absolute-configuration determinations. *J Appl Cryst* 33:1143–1148. doi:10.1107/S0021889800007184
57. Sheldrick GM (1997) *SHELXL97*, Program for the refinement of crystal structures. University of Göttingen, Germany
58. Wang J, Yang X, Cheng J, Xu Y, Liu B, Wang H, Zhang D (1996) Thermodynamic α -CH acidities of amino acid fragments in five co-ordinate bicycloazastannoxides in Me_2SO . *J Chem Soc Dalton Trans.* 3889–3891. doi:10.1039/dt9960003889
59. Wang J, Zhang Y, Xu Y, Wang Z (1992) Synthesis and characterization of pentacoordinate organo-tin(IV) complexes. *Heteroatom Chem* 3:599–602. doi:10.1002/hc.520030523
60. Matsubayashi G, Tanaka T, Nishigaki S, Nakatsu K (1979) Nuclear magnetic resonance studies of some *N'*-substituted pyridine-2-carbaldehyde adducts of *n*-butyltrichlorotin(IV) and the molecular structure of *n*-butyltrichloro(*N'*-phenylpyridine-2-carbaldehyde-*NN'*)tin(IV). *J Chem Soc Dalton Trans.* 501–505. doi:10.1039/dt9790000501
61. van Koten G, Noltes JG (1976) Novel chiral triorganotin halides. Stabilization of optically active tin centers by intramolecular coordination. *J Am Chem Soc* 98:5393–5395. doi:10.1021/ja00433a058
62. Holeček J, Nádvorník M, Handlir K, Lyčka A (1983) ^{13}C and ^{119}Sn NMR Study of some four- and five-coordinate triphenyltin(IV) compounds. *J Organomet Chem* 241:177–184. doi:10.1016/S0022-328X(00)98505-X
63. Willem R, Verbruggen I, Gielen M, Biesemans M, Mahieu B, Basu Baul TS, Tiekink ERT (1998) Correlating Mössbauer, solution and solid state ^{119}Sn NMR data with X-ray diffraction structural data of triorganotin 2-[(*E*)-2-(2-hydroxy-5-methylphenyl)-1-diazenyl]benzoates. *Organometallics* 17:5758–5766. doi:10.1021/om980504u
64. Boyd MR (1989) Status of the NCI preclinical antitumor drug discovery screen. *Principles and practice of oncology*, vol 3, pp 1–12. Lippincott, Philadelphia
65. Keepers YP, Pizao PR, Peters GJ, Ark-Otte JV, Winograd B, Pinedo HM (1991) Comparison of the sulforhodamine B protein and tetrazolium (MTT) assays for in vitro chemosensitivity testing. *Eur J Cancer* 27:897–900
66. Nath M, Yadav R, Gielen M, Dalil H, de Vos D, Eng G (1997) Synthesis, characteristic spectral studies and *in vitro* antimicrobial and antitumour activities of organotin(IV) complexes of Schiff bases derived from amino-acids. *Appl Organomet Chem* 11:727–736. doi:10.1002/(SICI)1099-0739(199709)11:9<727::AID-AOC639>3.0.CO;2-X
67. Ogwuru N, Khoo LE, Eng G (1998) A study of the antitumor activity of four dibutyltin(IV)-*N*-arylidene- α -amino acid complexes. *Appl Organomet Chem* 12:409–417. doi:10.1002/(SICI)1099-0739(199806)12:6<409::AID-AOC707>3.0.CO;2-F
68. Tian L, Sun Y, Qian B, Yang G, Yu Y, Shang Z, Zheng X (2005) Synthesis, characterization and biological activity of a novel binuclear organotin complex, $\text{Ph}_3\text{Sn}(\text{HL})\cdot\text{Ph}_2\text{SnL}$ L = 3,5-Br₂-2-OC₆H₂CH=NCH(*i*-Pr)COO. *Appl Organomet Chem* 19:1127–1131. doi:10.1002/aoc.968
69. Tian L, Sun Y, Yang G, Qian B, Shang Z (2005) Formation, structure and in vitro antitumour activity of a novel binuclear organotin complex. *Chin Chem Lett* 16:1584–1586
70. Casini A, Messori L, Orioli P, Gielen M, Kemmer M, Willem R (2001) Interactions of two cytotoxic organotin(IV) compounds with calf thymus DNA. *J Inorg Biochem* 85:297–300. doi:10.1016/S0162-0134(01)00215-X